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13. ABSTRACT (Maximum 200 Words)

Androgen ablation constitutes the most common therapy for the treatment of advanced prostate cancer. While initially effective at reducing tumor burden, most patients recur with androgen insensitive disease. There exists a clear need to augment the clinical efficacy of hormone-based therapies and immunotherapy of prostate cancer represents one such promising approach. The induction of apoptotic cell death in the prostate is accompanied by an inflammatory infiltrate comprised predominantly of activated T cells. This AA induced autoimmune-like response exerts limited anti-tumor activity in a murine prostate cancer model, but could be synergistic with CTLA-4 blockade that promotes the development of autoreactive T cell. In the past year, we have established stable clonal cell lines expressing the CD80 molecule that increases the immunogenicity of the tumor cells, set up the tumor models and performed dose-titration experiments and examined the effects of castration on existing tumors.

14. SUBJECT TERMS
Prostate cancer, immunotherapy, androgen ablation, mouse model, clinical trial

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Table of contents

Introduction

Androgens are required for the normal growth, development and function of the prostate gland and also support the growth of prostate neoplasms. Since the early 1940s, androgen ablation (AA) therapy has been a standard treatment for advanced prostate cancer after Huggins and Hodges(1) made the observation that prostate tumors are partially responsive to androgen withdrawal. AA remains a palliative approach as it does not totally eliminate all the prostate cancer cells. While initially effective at reducing tumor burden, most patients eventually develop disease refractory to androgen withdrawal. The hormone refractory stage of prostate cancer represents the terminal stage of the disease as treatment options are limited and the median survival for such patients is approximately 1 year.

Androgens exert potent immunosuppressive effects and it has been shown that patients with advanced prostate cancer have dysfunctional cell-mediated immunity characterized by the predominance of a Th2 cytokine profile(2). It was also shown that the dendritic cells (DC) from prostate cancer patients were functionally impaired and were less potent stimulators in an allogenic mixed leukocyte reaction(3). Castration of mice stimulates B and T lymphopoiesis, thymic and bone marrow hyperplasia(4). Immunotherapeutic approaches for the treatment of prostate cancer could be affected by the AA since the loss of androgens may influence the nature of the host immune response. This study aims to identify the effects of AA on anti-tumor immunity and to determine the mechanism behind any effects.

Body

This progress report describes the activities on the grant proposal from September 2003 to September 2004. During this year, no funding was available as the P.I moved from Loyola University Chicago to USC and it took exactly 1 year for Loyola University Chicago to relinquish the grant. Without funding, progress was delayed. A new graduate student, Yi Ting Koh, who joined the lab in March 2004, has been assigned to this project and trained in the castration procedure.

Specific Aim 1: Task 2: Confirm uniform expression of CD80 in B16 and TRAMP-C2 stable transfectants

B16 and TRAMP-C2 cells lines were stably transfected with CD80 and stable clones selected using limiting dilution. To ensure uniform expression of CD80, the CD80-expressing clones were further purified by cell sorting.

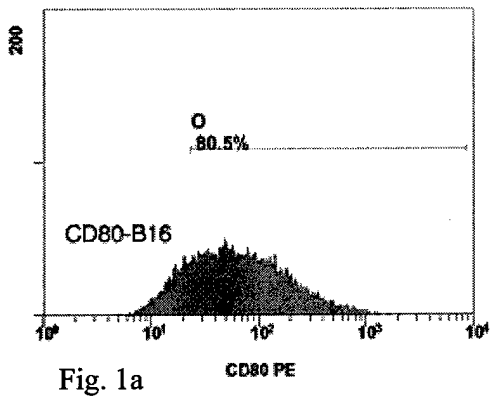

Fig. 1a

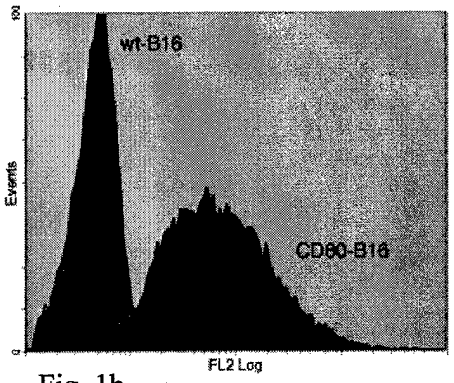

Fig. 1b

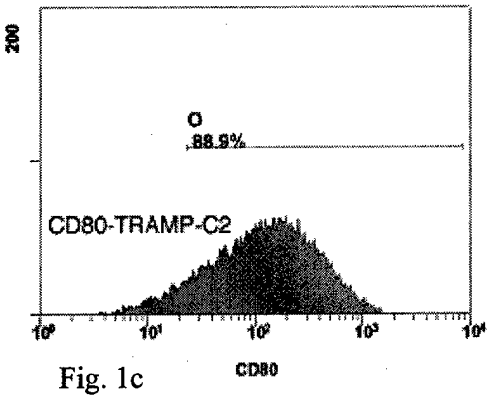

Fig. 1c

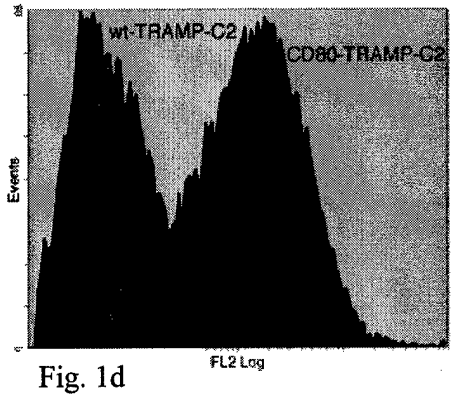

Fig. 1d

Figure 1. Analysis of CD80 expression on (a) B16 and (c) TRAMP-C2 stably transfected clones by flow cytometry and overlay histograms comparing expression of CD80 on transfected (b) B16 and (d) TRAMP-C2 versus wild-type (wt).

Body (continued)

Specific Aim 1: Task 3 : Perform titration experiments for tumor induction for B16 tumor cell line

6-8 weeks old male C57BL/6 mice (n=5) were injected subcutaneously with either B16 or CD80-expressing B16 tumor cells with tumor doses ranging from $1x10^5$ to $5x10^6$. Tumor growth was measured over time to determine if the immunogenic CD80-B16 tumor would regress over time. Unfortunately, at all the tumor doses, there was no regression of the CD80-B16, although the CD80-B16 tumors had a slower growth rate than the wild-type B16 tumors.

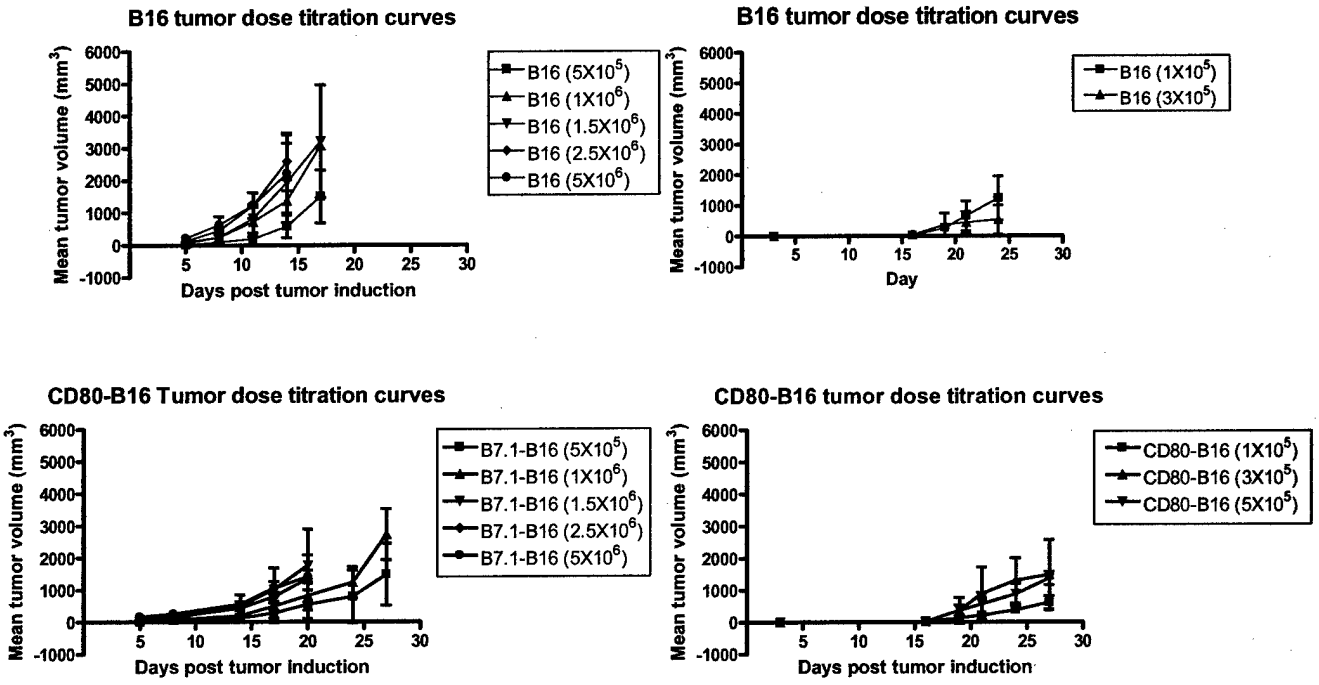

Figure 2. Tumor dose titration curves for B16 and CD80-B16 cell lines.

Body (continued)

Specific Aim 1: Task 6 : Determine effects of AA on established B16 and CD80-B16 tumors

6-8 weeks old C57BL/6 mice were injected with $5x10^4$ B16 or CD80-expressing B16 cells and castrated or sham-castrated 7 days later. From the data obtained, there was an obvious reduction in growth rate in the CD80-expressing B16 cells (r=0.401, p=0.0025). The effects of castration on tumor growth were not statistically significant. In the CD80-B16 cells, castration seemed to result in a slight increase in the tumor growth (r=0.31, p=0.082). However, in the B16 cells, castrated mice had a slower growth rate then the sham-castrated mice (r=0.22, p=0.149).

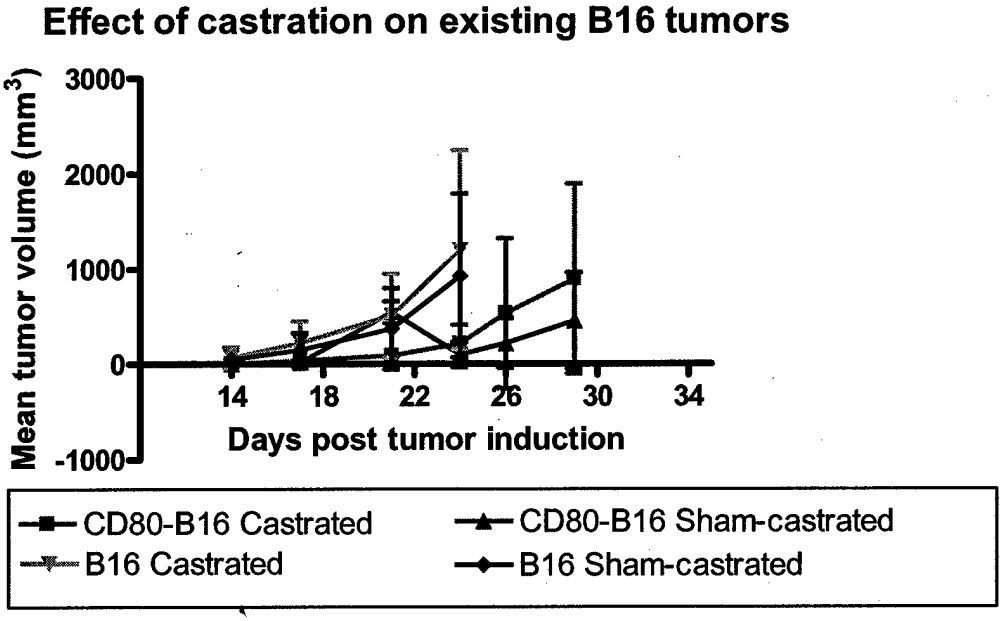

Figure 3. Effect of castration on existing B16 tumors

Body (continued)

Specific Aim 1: Task 6 : Determine effects of AA on established B16 and CD80-B16 tumors

6-8 weeks old C57BL/6 mice were injected with $1x10^6$ B16 or CD80-expressing TRAMP-C2 cells and castrated or sham-castrated 7 days post tumor induction. From the data obtained, there was a statistically significant increase in the tumor size in the castrated animals bearing both TRAMP-C2 (p=0.0251) and CD80-TRAMP-C2 tumors (p=0.0196). At the moment, we are in the process of carrying out immunohistochemistry on the tumors in order to examine if AA results in increase tumor infiltrating lymphocytes that account for the larger tumor mass observed.

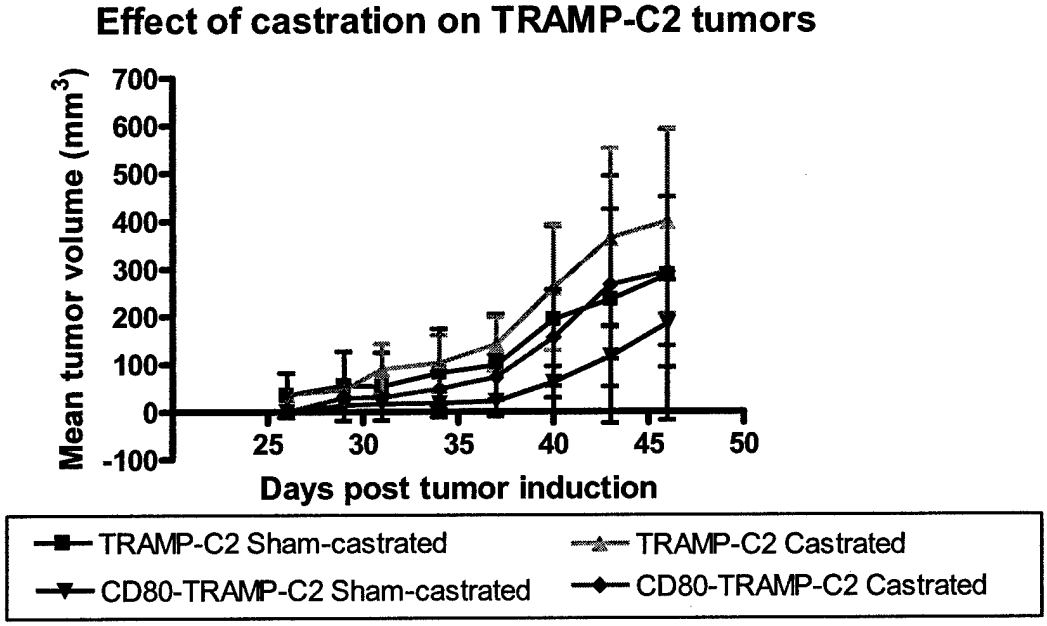

Figure 4. Effect of castration on existing TRAMP-C2 tumors.

Key research accomplishments

1. Obtained stable clones of CD80-expressing B16 and TRAMP-C2 cell lines
2. Performed tumor dose titration experiments for B16 and CD80-B16 cell lines
3. Investigated effects of castration on existing B16, CD80-B16, TRAMP-C2 and CD80-TRAMP-C2 cell lines.

Reportable outcomes

Manuscripts, abstracts, presentations:

One review article

1. Koh YT, García-Hernández ML, Kast WM.
   Tumor immune escape mechanisms in Drug resistance in malignant disease editor, BA. Teicher. (*in press*) 2004

Patents and licenses applied for and/or issued:

None

Degrees obtained that are supported by this award:

None

Development of cell lines, tissue or serum repositories:

Two stable clones

1. CD80-B16 cell line
2. CD80-TRAMP-C2 cell line

Informatics such as databases and animal models, etc:

None

Funding applied for based on work supported by this award:

None

Employment or research opportunities applied for and/or received on experiences/training supported by this award:

Dr. García-Hernández was awarded a fellowship from the Department of Defense #PC041078

Conclusions

We have obtained stable clonal cell lines of B16 and TRAMP-C2 tumors that express high levels of CD80. AA alone was not able to inhibit the growth of existing immunogenic or non-immunogenic B16 tumors and actually led to an increase the size of TRAMP-C2 tumors. We hope the immunohistochemistry analysis of the tumors will enable us to understand if the increase in tumor mass might be due to increase in tumor infiltrating lymphocytes. Work is also currently underway in order to determine if AA results in the generation of more potent antigen-presenting cells that could increase the efficacy of immunotherapy strategies and if AA results in higher vaccine-induced immune responses against TRAMP-C2 tumors.

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